International Journal of Agriculture and Biosciences 2026 15(2): 489-495.
https://doi.org/10.47278/journal.ijab/2025.207

RESEARCH ARTICLE
eISSN: 2306-3599; pISSN: 2305-6622

Diversity and Seasonal Prevalence of Fish Parasites in Freshwater Aquaculture Systems of Charsadda, KP, Pakistan

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ABSTRACT

Aquaculture is a rapidly growing industry and a vital source of protein worldwide. However, parasitic infection/infestation poses significant challenges to its sustainability, particularly in developing countries like Pakistan. This study examines the diversity, prevalence, and seasonal occurrence of parasites in fish farms in Charsadda, Khyber-Pakhtunkhwa, Pakistan. Parasites were diagnosed through microscopic examination of gills, skin, fins, and blood smears, and identified morphologically using standard taxonomic keys. A total of 500 fish specimens were collected from seven farms, revealing an overall infection/infestation rate of 49.2%. Among the identified parasites, *Lernaea spp*. exhibited the highest prevalence (14.2%), while *Trypanosoma spp*. showed the highest mean intensity (8.43 parasites per infected fish). Seasonal trends were evident, with peak parasite prevalence occurring during the warmer months (spring and summer), particularly for ectoparasites like *Lernaea spp*. and *Argulus spp*. Chi-square analysis revealed significant differences in parasite prevalence across fish species, emphasizing the severe impact of *Trypanosoma spp*. on fish health. Farm-specific parasite profiles were also noted, indicating that environmental factors, water quality, and farming practices influence the distribution of parasites. These findings underscore the critical role of environmental factors, water quality, and the distribution of farming practices. Effective management strategies, including regular monitoring and enhanced biosecurity, are crucial for mitigating parasitic threats and promoting sustainable aquaculture in the region.

Keywords: Parasites; Aquaculture; Infection; Infestation; Prevalence; Charsadda; Pakistan.

Article History
Article # 25-348
Received: 17-Jun-25
Revised: 15-Sep-25
Accepted: 06-Oct-25
Online First: 06-Dec-25

INTRODUCTION

Aquaculture is one of the world's most commercially significant sectors and fish are one of humanity's most valuable and nutritional resources (Dar et al., 2014). With the rising global demand for low-cost protein sources, fish are receiving considerable attention. Pakistan, primarily an agricultural country, is rich in natural water resources, with freshwater and marine resources serving as the primary bases for aquaculture (Jarwar, 2008). Aquaculture Is the fastest-growing food industry and is predicted to be the future primary protein source for humans. Its

Cite this Article as: Ullah I, Baset A, Nouh NA, Abdulrahman WMA, Mohamed HM, Khan MA, Sunny F, Siddique A, Shams F and Hussain H, 2026. Diversity and seasonal prevalence of fish parasites in freshwater aquaculture systems of Charsadda, KP, Pakistan. International Journal of Agriculture and Biosciences 15(2): 489-495. https://doi.org/10.47278/journal.ijab/2025.207

A Publication of Unique Scientific Publishers

development is closely related to growing environmental awareness and the issues associated with industrial farming. In the commercialization of the aquaculture industry, parasites and health management practices have emerged as primary concerns (Abbas et al., 2023).

Disease outbreaks in fish remain the most devastating challenge for aquaculture production. Many freshwater fish species are seriously afflicted with various parasites, which results in high fish mortality and reduced aquaculture productivity, thereby negatively impacting the economy (Shafiq et al., 2023). Infections caused by various fish parasites can hinder the development of aquaculture systems. The significance of fish parasites is directly connected to the significance of fish health, as the host's tissues or gut contents are the primary sources of nutrition for these parasites, which, under favorable conditions, reproduce rapidly (Iwanowicz, 2011). The presence of parasites in fish may lead to mechanical, physiological, and reproductive damage. Fish are affected worldwide by a wide range of parasitic diseases, which cause mortality either directly through tissue damage and organ failure or indirectly by weakening the host immune and increasing its susceptibility to secondary infections (Sures & Nachev, 2022). Moreover, the presence of parasites in aquatic species serves as an indicator of the degradation of aquatic habitats (Giari et al., 2022). Infectious parasites infecting fish have both direct and indirect impacts on human health, since both living parasites and pathogens that have already died and remain in fish tissue can cause allergic reactions in humans (Iwanowicz, 2011).

Pakistan's geographic position endows it with diverse climatic conditions and an abundance of marine, brackish, and freshwater resources that can be utilized for economically efficient and environmentally sustainable aquaculture. Since the early 1970s, inland fisheries have been shifting from extensive to semi-intensive systems through the development of fish farms and the adoption of artificial spawning techniques for restocking natural and commercial fish production systems. The fisheries sector in Pakistan provides direct employment to 400,000 individuals and indirectly supports an additional 600,000 people (Humayun & Zafar, 2014). However, despite significant growth, a critical need for further advancement and strategic planning remains, as the overexploitation of marine resources has created pressures that necessitate stricter regulations and sustainable practices. Although Pakistan's aquaculture sector holds immense potential to enhance the overall fisheries industry, its actual progress has been relatively slow; focused efforts are needed to modernize techniques, improve infrastructure, and promote environmentally sustainable practices that align with global standards (Afzal et al., 2023).

Parasites threaten farmed fish, resulting in significant economic losses and posing health risks to humans through zoonotic infections such as those caused by helminths (nematodes, cestodes, trematodes, and acanthocephalans) (Ziarati et al., 2022). For instance, anisakid nematodes are among the most common fish-borne zoonotic parasites, causing human infection via the consumption of third-stage larvae in undercooked fish hosts. Moreover, the nematode *Capillaria philippinensis* may also be transmitted by ingesting infected fish, although freshwater fish are the typical second intermediate hosts (Betson et al., 2020).

This study is crucial, as it highlights the widespread prevalence of parasitic infection in fish farms in Charsadda, Khyber-Pakhtunkhwa, Pakistan, thereby posing significant threats to aquaculture productivity. By identifying the key parasitic species affecting farmed fish and analyzing their distribution, mean intensity, and relative density, this research provides valuable insight for improved management strategies.

MATERIALS & METHODS

Study Area

A total of 500 fish samples were randomly collected from Charsadda, Khyber Pakhtunkhwa, Pakistan. Charsadda is a district in the Peshawar Division of Khyber Pakhtunkhwa province. It lies between 34.1454° N and 71.7307° E. Charsadda was selected because of its extensive freshwater resources (rivers, canals) and dense network of fish farms make it a representative and important site for studying parasitic infection/infestation in aquaculture systems of Khyber Pakhtunkhwa. Map of the study area is shown in Fig. 1.

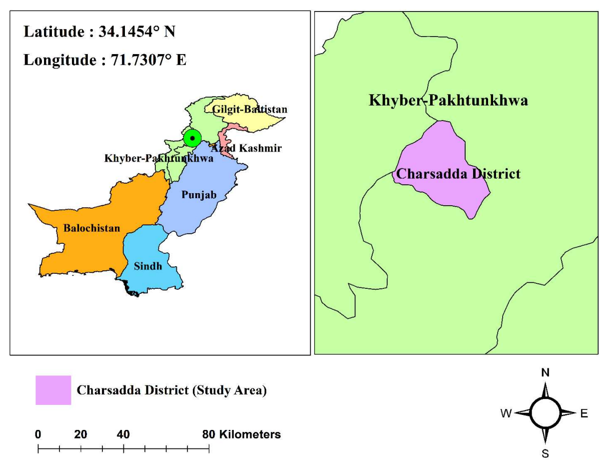

Fig. 1: Map of Pakistan and Khyber-Pakhtunkhwa showing Study Area at District Charsadda.

Fish Collection

Fish were randomly collected from each farm to ensure a representative sample of the entire population. This sampling method guaranteed that the selected fish accurately reflected the characteristics of the larger population (Nguka, 2017).

Fish Processing

The collected fish samples from the study sites were transported to the laboratory in sealed bags. Assistance was provided by local fishermen and experts to ensure the accurate collection of fish species from the sampling area (Hamayoon et al., 2024). To prevent cross-contamination of ectoparasites, surgical gloves were worn during the collection and handling of the fish. After collection, each fish sample was preserved by injecting 10% formalin through the mouth and anus (Motomura et al., 2013). Morphometric measurements were then taken by following the methodology suggested by Mirza and Sandhu (2007).

Ectoparasites Processing

For collection and identification of ectoparasites, fish samples were thoroughly examined with the naked eye. Each sample was then investigated using a magnifying glass and a binocular microscope. Ectoparasite examination was performed following the method described by Fernando (1972), which involved gently scraping the skin, fins, and, gills of each fish to collect parasites for microscopic analysis. . Specific fish parts for the parasites such as eyes, nostrils, mouth, and fins were examined for the presence of parasites. Gills were carefully removed through scissors and placed in a Petri dish with the help of forceps; visible parasites such as *Lernaea spp*. were removed and preserved in 70% ethanol. Microscopic ectoparasites were collected using the scraping technique following Yusni and Rambe (2019).

Blood Parasites Processing

Blood samples were collected from each fish sample using 5cc Medi-Aqua syringes for studying the blood parasites. Blood was drawn from the caudal vein and heart. The collected blood was then stored in EDTA tubes to prevent coagulation. Thin blood smears were prepared from the freshly collected blood samples. The smears were dried in the air before being fixed in 100% methanol. The slides were stained using phosphate-buffered Geimsa and examined under a microscope with a 100X oil immersion objective (Alhayali et al., 2023).

Statistical Analysis

The prevalence, mean intensity, and relative density of infestation/infection were calculated by the formulas: Prevalence percentage (P%) = Number of host fish infected x 100 / number of host fish studied, Mean Intensity (MI) = Total number of parasites recovered/ Total number of infected host examined, Relative Density (RD) = Total number of parasites recovered / Total number of hosts examine. Prevalence data were analyzed using the Chi-square test to assess the significant differences of parasites prevalence. Mean intensity and relative density were analyzed using ANOVA followed by Tukey's HSD test for pairwise comparisons. All analyses were performed using SPSS v26.

Data Visualizations and Mapping

Microsoft Excel 2019 was used for data visualization and to create illustrative graphs. The study area map was prepared using ArcGIS, while the methodological framework was designed with EdrawMax, outlined in Fig. 2.

RESULTS

A total of 500 fish specimens were collected from seven farms Mohmand Fish Farm, Khyber Fish Farm, Shabara Fish Farm, Hayat Shaheed Fish Farm, Nisatta Fish Farm, Mujahid Fish Farm, and Rajjar Fish Farm in Charsadda Khyber-Pakhtunkhwa, Pakistan. These specimens were sampled over a year, representing all four seasons: Spring, Summer, Autumn, and Winter. Of these, 246 fish were found to be affected with various ectoparasites and blood parasites, resulting overall infection/infestation rate of 49.2%. The overall findings are given below:

The distribution of 500 fish samples among the farms was as follows: 62 from Mohmand Fish Farm, 81 from Khyber Fish Farm, 90 from Shabara Fish Farm, 70 from Hayat Shaheed Fish Farm, 53 from Nisatta Fish Farm, 89 from Mujahid Fish Farm, and, 55 from Rajjar Fish Farm, a detailed breakdown of the sampling from each farm is presented in Table 1.

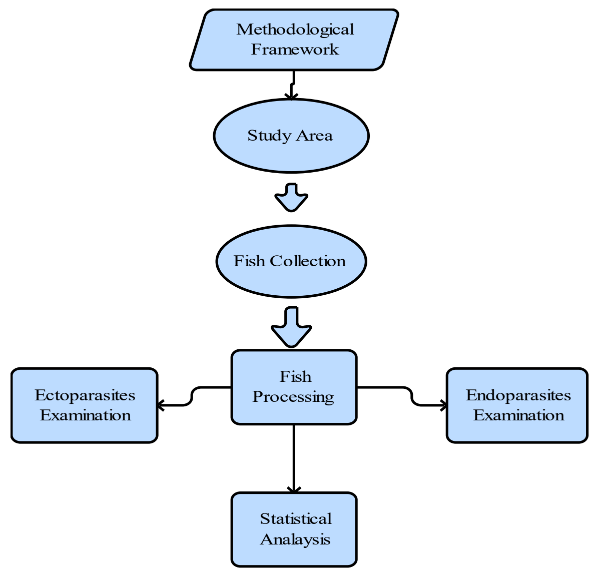

Fig. 2: Methodological Framework of the article.

Table 1: Number of fish sampled from each fish farm in Charsadda

S. No	Fish farms	Numbers of fish	No of positive fish	% Infection /infestation
1	Mohmand Fish Farm	62	31	50.0
2	Khyber Fish Farm	81	39	48.1
3	Shabara Fish Farm	90	47	52.2
4	Hayat Shaheed Fish Farm	70	35	50.0
5	Nisatta Fish Farm	53	25	47.2
6	Mujahid Fish Farm	89	44	49.4
7	Rajjar Fish Farm	55	25	45.5

The prevalence of different parasitic species was as follows: Lernaea spp. (Anchor worm) was the most prevalent, infecting 71 fish with a prevalence rate of 14.2%. Argulus spp. (Fish Lice) was found in 58 fish, showing a prevalence rate of 11.6%. Ichthyophthirius multifiliis (White Spot) infected 37 fish, accounting for a prevalence rate of 7.4%. Gyrodactylus spp. (Skin Flukes) was observed in 32 fish, with a prevalence rate of 6.4%. Dactylogyrus spp. (Gill Flukes) was detected in 27 fish, representing a prevalence rate of 5.4%. Lastly, Trypanosoma spp. (Blood parasites) infected 21 fish, showing a prevalence rate of 4.2%. Detailed data are presented in Table 2 and Fig. 3.

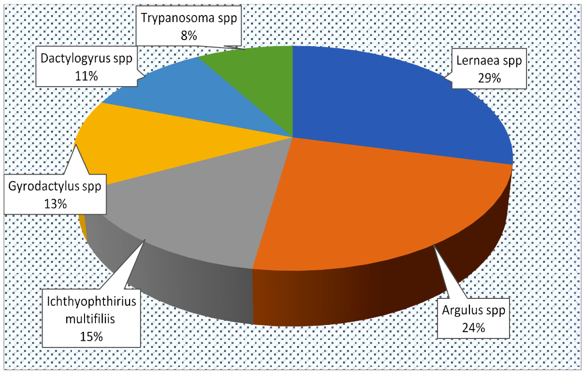

Fig. 3: Prevalence percentage of parasitic infections/infestations in the sampled fish population.

Mean Intensity (MI)

The mean intensity (MI) represents the average number of parasites per infected fish. The highest mean intensity was recorded for *Trypanosoma spp*. (8.43 parasites per infected fish), indicating a severe parasite burden among affected individuals. *Dactylogyrus spp*. (1.84) and *Ichthyophthirius multifiliis* (1.78) also exhibited relatively high intensities. In contrast, *Argulus spp*. showed the lowest mean intensities (1.36), suggesting that affected fish harbored fewer parasites on average. Detailed data is provided in Table 2.

Relative Density (RD)

Relative density provides insight into the overall distribution of parasites within the sampled fish population. The highest relative density was observed for *Trypanosoma spp*. (0.354 parasites per total fish sampled), followed by *Lernaea spp*. (0.218) and *Argulus spp*. (0.158). The lowest relative density value was recorded for *Dactylogyrus spp*. (0.096), indicating its limited occurrence among the examined fishes. Detailed data is presented in Table 2.

Parasite Prevalence (Chi-square Analysis)

Chi-square analysis revealed significant differences in parasite prevalence among the parasite species (χ^2 =45.9, df = 5; P<0.001). Lernaea showed the highest prevalence (14.2%), whereas *Trypanosoma* exhibited the highest mean intensity (8.43). This indicates that parasite species affects the sampled population differently, emphasizing the need for targeted monitoring and management strategies.

Variations in Parasites Mean Intensity (ANOVA)

Statistical analysis revealed significant differences in mean intensity (MI) of parasites across species (F=123.45, P<0.0001). Post-hoc comparison using Tukeys HSD test showed that *Trypanosoma spp.* had a significantly higher mean intensity compared to all other species (p<0.05). No significant differences were observed among *Lernaea, Argulus, Icthyophthirius multifiliis, Gyrodactylus, Dactylogyrus* (P>0.05).

Farm-specific Parasitic Profile

Table 3 provides a detailed overview of the parasites reported in this study. This reveals farm-specific parasite profiles, suggesting that environmental factors, water quality, and farming practices play a significant role in parasite distribution. This information is critical for developing targeted management strategies to control parasitic infections in aquaculture systems.

Farms' Water and Air Profile

The water quality parameters of the farms surveyed revealed seasonal variations that generally align with the needs of cultured fish species such as *Labeo rohita, Catla catla, Hypophthalmichthys molitrix, Ctenopharynodon idella,* and *Nile tilapia*. The pH levels remain within a slightly alkaline to neutral range (6.7-7.9), providing suitable conditions for fish health. Air temperature peaks in summer (up to 41°C) which accelerates parasite life cycles and declines in winter (as low as 14°C). While water temperature follows a similar seasonal pattern as air temperature, but with slightly smaller fluctuations, ranging from 13°C in winter to 37°C in summer. Dissolved oxygen (DO) levels exhibit notable seasonal variations, being lowest in summer (3.4-4.8mg/L) due to higher water temperature and highest in winter (up to 7.7mg/L) as cooler water enhances oxygen solubility. Despite these fluctuations, most farms maintain DO levels above 3.0mg/L, which is critical for fish survival. However, during summer, some farms experience elevated water temperatures which reduced dissolved oxygen levels. This decrease in DO can stress the fish population and impair their immune function. Additionally, nutrient pollution from agriculture runoff can also lead to eutrophication, supporting the growth of intermediate hosts like copepods, leeches etc. which are essential for many parasites. Detail data is presented in Table 4.

Seasonal Variations in Parasite Prevalence

The seasonal distribution of parasites shows significant differences across farms. The data showed that parasitic prevalence peaked during the summer months, with higher infestations/infections of *Lernaea spp*., *Argulus spp*., and *Trypanosoma spp*. Unlike other parasites that are strongly temperature dependent, thriving in spring and summer but declining sharply in autumn and winter, *Trypanosoma spp*. Maintained consistent prevalence throughout the year. This seasonal trend underscores the critical role of environmental factors, such as water temperature, in driving parasite proliferation. Farms should therefore use targeted parasite control measures during peak seasons, especially summer, while maintaining control against *Trypanosoma spp.* throughout the year due to its unique adaptability. These insights highlight the need for strategic, season-specific management practices to mitigate parasite-related challenges effectively. The seasonal prevalence of parasites can be seen in Table 5, while parasites recovered in Table 6.

Table 2: Different Statistical Values (P%, MI, RD)

Parasite species	Number of infected fish	Total parasites recovered	Prevalence %	Mean intensity (MI)	Relative density (RD)
Lernaea	71	109	14.2	1.54	0.218
Argulus	58	79	11.6	1.36	0.158
Icthyophthirius multifiliis	37	66	7.4	1.78	0.132
Gyrodactylus	32	59	6.4	1.84	0.118
Dactylogyrus	27	48	5.4	1.78	0.096
Trypanosoma	21	177	4.2	8.43	0.354
Total	246	538	100		

Table 3: Specific Parasites Reported from Specific Farms

Water Bodies	Parasites
Mohmand Fish farm	*Trypanosoma* spp. & *Argulus* spp.
Khyber Fish Farm	*Argulus Spp. Larnaea spp.* & *Gyrodactylus spp.*
Shabara Fish Farm	*Trypanosoma spp. Larnaea spp. Argulus spp.* & *Gyrodactylus spp.*
Hayat Shaheed Fish Farm	*Trypanosoma* spp. & *Icthyophthirius multifiliis.*
Nisatta Fish Farm	*Icthyophthirius multifiliis, Larnaea* spp. & *Dactylogyrus spp.*
Mujahid Fish Farm	*Larnaea* spp. *Argulus* spp. & *Dactylogyrus*
Rajjar Fish Farm	*Larnaea spp. Trypanosoma spp.* & *Gyrodactylus spp.*

Table 4: Seasonal Variations Among All Selected Farms

Fish farm	Seasons	pH	Air temperature	Water temperature	DO mg/L
Mohmand Farm	Spring	7.1	27	20	4.3
	Summer	7.3	37	29	4.1
	Autumn	7.5	23	22	4.4
	Winter	7.8	17	15	4.7
Khyber Farm	Spring	7.2	25	21	4.9
	Summer	7	39	30	4.8
	Autumn	7.3	26	21	4.9
	Winter	7.7	15	13	5.1
Shabara Farm	Spring	7	25	22	4.1
	Summer	7.3	31	29	4.3
	Autumn	7.4	23	21	5.3
	Winter	7.9	14	13	6.1
Hayat Farm	Spring	7.2	26	24	6.3
	Summer	7.3	36	31	4.6
	Autumn	7.3	25	23	7.2
	Winter	7.7	19	17	7.7
Nisatta Farm	Spring	7.6	29	25	6.1
	Summer	7.4	39	35	3.9
	Autumn	7.5	25	22	7.1
	Winter	7.9	16	14	7.3
Mujahid Farm	Spring	6.9	27	26	3.9
	Summer	7	40	37	3.4
	Autumn	7.3	31	27	6.1
	Winter	7.5	21	20	7
Rajjar Farm	Spring	6.7	27	26	3.2
	Summer	6.6	41	36	4.1
	Autumn	7	26	24	5.1
	Winter	7.3	18	15	6.6

Table 5: Seasonal Prevalence Percentage of Each Parasite Round the Year

Parasites species	Summer %	Spring %	Autumn %	Winter %
Lernaea	26.4	36.8	18.4	5.6
Argulus	16.8	30.4	8.8	7.2
Icthyophthirius multifiliis	15.2	23.2	9.6	4.8
Gyrodactylus	11.2	23.2	7.2	5.6
Dactylogyrus	8.8	15.2	10.4	4
Trypanosoma	34.4	46.4	31.2	29.6
Total	100	100	100	100

Table 6: Number of Parasites Recovered Each Season

Parasite species	Spring	Summer	Autumn	Winter	Total
Lernaea	33	46	23	7	109
Argulus	21	38	11	9	79
Icthyophthirius multifiliis	19	29	12	6	66
Gyrodactylus	14	29	9	7	59
Dactylogyrus	11	19	13	5	48
Trypanosoma	43	58	39	37	177

DISCUSSION

The findings of this study reveal a high prevalence of parasitic infection/infestation in freshwater farms in Charsadda, Khyber-Pakhtunkhwa. The dominance of *Lernaea spp*. and *Argulus spp*. suggests that environmental factors such as water and stocking density play critical roles in parasite distribution. The high mean intensity of *Trypanosoma spp*. indicates a severe burden on infected fish, potentially leading to reduced growth and increased mortality. With an overall infection incidence of 49.2%, the current study showed that parasitic infections were quite prevalent in fish farms located in the Charsadda district of Khyber Pakhtunkhwa. Our results demonstrated that parasite infestations are a serious threat to aquaculture systems' production, particularly when they are brought on by ectoparasitic copepods like *Lernaea* spp. Our findings that *Lernaea* dominated prevalence aligns with Bilal et al. (2021), who similarly documented high infestation in *Catla catla* compared to zero in *Oreochromis niloticus*, and linked such differences to farm-specific water quality and density factors.

Abbas et al. (2023), our findings indicate that while some species (such as *Oreochromis niloticus*) seem resistant, species like *Catla catla* are more vulnerable (41.7% prevalence). Similarly, gastrointestinal helminths such as *Capillaria pterophylli* have recently been reported in Indus River Fishes, *O. niloticus* showing 7.5% prevalence, underscoring that even relatively resistant species are not entirely parasite-free (Rehman et al., 2025). In a similar study conducted in the tehsil Okara Punjab Pakistan, 26 parasite species were identified, including, protozoans, trematodes, and monogeneans, from various fish species. The diversity underscores the widespread nature of parasitic infections in freshwater systems across Pakistan (Shafiq et al., 2023). Furthermore Ahmed et al. (2007) reported high infection intensities for different parasites, with values ranging from 1.0 for cestodes to 14.36 for acanthocephalans per infected fish. Such high intensities may be attributed to environmental conditions that favor the life cycles of these parasites, such as warm water temperatures and organic pollution. A similar pattern was observed in a recent study where *Lernaea* prevalence rearched 37.5% in larger *Labeo rohita* and dropped to zero by April, highlighting strong seasonal influences on parasite load (Khan & Zeeshan, 2024).

In another study Tayyab *et al*. (2017) examined fishes, and reported species of different parasites, from the Indus

River D.I Khan, including *Saprolegnia*, microsporidia, *Lernaea cyprinacea*, and *Sphaerospora* species. Similarly, Jalilpoor et al. (2006) reported parasitic infections in *Labeo rohita* with *Saprolegnia* spp. being the primary causative agent. These findings highlight the widespread occurrence of parasitic infections in freshwater fish across different regions of Pakistan. Ayaz et al. (2013) conducted a study between July and October 2010. The overall prevalence of parasites in freshwater fishes was 41.67%. The identified species included: *Rhobdocorna magna* (7.5%), *Camallanus* (11.7%), *Senga taunsaensis* (6.67%) and *Helicometra fasciata*, (15%).

Similar research studies in other parts of the world showed that parasite diseases not only harm fish health but also cause zoonotic issues, which are becoming more significant as aquaculture spreads throughout the world (Abd-Elrahman et al., 2023).

Environmental factors that seem to have a significant impact on parasite reproduction and transmission include water temperature and dissolved oxygen. Our findings that infestation rates are higher in the summer are consistent with previous results from South Tamil Nadu, India and Pakistani inland fisheries (Abbas et al., 2023). These seasonal fluctuations highlight how climate influences host susceptibility and parasite life cycles. Additionally, recent research from Khyber Pakhtunkhwa Jarar et al. (2023) has emphasized the importance of appropriate stocking and water management techniques in reducing parasite outbreaks. Our work benefits from methodological insights provided by studies on parasite-host relationship In the Arabian Gulf Khalil & Abdelkader (2014).

Similarly, global evidence highlights host-related differences in parasite diversity Silva et al. (2025) reported that *Astyanax bimaculatus* harbored a higher diversity and abundance of parasites compared *to Psalidodon fasciatus,* despite both species occurring sympatrically in Northeastern Brazil. Such findings emphasize that parasites burdens are influenced not only by environmental conditions but also by intrinsic host traits such as size and abundance. Similarly, Zaharieva et al. (2025) reported five parasites species, including three pathogenic ones, in common carp from the Danube River, Bulgaria, with two new host records. This highlights that parasite diversity and pathogenic infections are a global concern, emphasizing the need for monitoring parasites worldwide.

Conclusion

This study provides valuable insights into the prevalence and intensity of parasitic infections/infestations in fish farms in Charsadda, Khyber-Pakhtunkhwa Pakistan. With nearly half of the sampled fish affected by various parasites, the economic and ecological implications are substantial. The dominance of *Lernaea spp*. and *Argulus* spp. along with the high mean intensity of *Trypanosoma spp*. suggests that environmental conditions and farming practices significantly influence parasite prevalence. Addressing these challenges requires a multi-faceted approach, including enhanced water quality management, regular health monitoring, and strict biosecurity protocols. Future research should focus on longitudinal studies to assess seasonal variations in parasite dynamics and explore innovative control measures. By implementing targeted interventions, Pakistan's aquaculture sector can achieve sustainable growth, ensuring food security and economic prosperity for local communities.

DECLARATIONS

Funding: This research did not receive any specific grant or funding from external agencies, institutions, or organization. The work was conducted without financial support, and no funding was granted for this study.

Acknowledgement: This research work was facilitated by the Department of Zoology at Bacha Khan University Charsadda Pakistan and Department of Chemical and Life Sciences Qurtuba University Peshawar Pakistan.

Conflict of Interest: The current study's authors stated that they had no conflicts of interest.

Data Availability: The data presented in this study can be accessed upon a fair request to the corresponding author.

Ethics Statement: Not applicable to our article.

Author's Contribution: Inayat Ullah conducted sampling, parasite identification, data analysis, and wrote the manuscript. Abdul Baset assisted with data analysis and manuscript revision. Nehal A. Nouh, Wafaa M. A. Abdulrahman, and Hagar M. Mohamed contributed to parasite diagnosis and critical review. Muazzam Ali Khan and Farhat Sunny provided aquaculture and fish-health expertise and reviewed the results. Aisha Siddique and, Farrah Shams offered microbiological input. Hamza Hussain assisted in field sampling and data collection. All authors contributed to the interpretation of findings and approved the final manuscript.

Generative AI Statement: The authors declare that no Gen AI/DeepSeek was used in the writing/creation of this manuscript.

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